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*J Immunol* 2010; 185:2737-2746; Prepublished online 26 July 2010; doi: 10.4049/jimmunol.1000758

http://www.jimmunol.org/content/185/5/2737

Supplementary Material

http://www.jimmunol.org/content/suppl/2010/07/27/jimmunol.1000758.DC1

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TLR-Mediated Loss of CD62L Focuses B Cell Traffic to the Spleen during Salmonella typhimurium Infection

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B cells as Ag presenters and Ab producers are a major component of the adaptive immune response, however, they also have the ability to respond to pathogens in an innate fashion. They do this by recognizing structurally conserved pathogen ligands through TLRs expressed at the cell surface and in the endosomal compartment. Murine B cells are known to express TLR1–9 at the mRNA level (1), although there is differential expression in certain subsets. When stimulated through these receptors in vitro, B cells are induced to proliferate and differentiate into Ab-secreting plasma cells in a T cell-independent manner (2), whereas in vivo responses to T-dependent Ags also require TLR signaling in B cells for optimal Ab production (3, 4). TLR stimulation of B cells also induces upregulation of cell-surface MHC class II and costimulatory molecules (1), enhancing their Ag-presenting capacity, and secretion of cytokines such as IL-6, IL-10 and IFN-γ (1), allowing the regulation of helper and regulatory T cell responses (5). Therefore, the rapid innate response of B cells to pathogens via TLR stimulation has a direct impact not only on the developing adaptive B cell response, but also on the magnitude and phenotype of the Th cell response.

To study TLR-dependent B cell responses in vivo, we made use of the murine infection model of typhoid fever, Salmonella enterica serovar Typhimurium. Systemic infection by this intracellular Gram-negative bacterium, which resides predominantly in macrophages, results in the development of strong Th1 and Ab responses (6, 7). Early in infection, innate macrophage responses are required to control bacterial growth (8), whereas specific Th1-associated cytokines (IFN-γ, TNF-α) are also crucial (9–11). The necessity of TLR signaling for clearance of Salmonella infection is well characterized. C3H/HeJ mice that lack TLR4 expression and so cannot respond to LPS are highly susceptible to Salmonella infection (12). TLR4 is thought to be important early in infection for cytokine production and killing of bacteria, whereas TLR2 (which recognizes bacterial glycolipids and lipopeptides) plays a role later (13). The lack of the MyD88 adaptor protein during the primary immune response to an attenuated strain of Salmonella typhimurium results in increased bacterial load, but these mice are able to clear the infection, albeit with delayed kinetics (14, 15). These mice appear to have impaired IL-12 production and reduced Th1 responses (16) and may in fact mount a skewed Th2 response (14).

The specific role of B cells in the S. typhimurium infection model has also been studied (17–19). B cells appear dispensable for the primary immune response to attenuated strains of Salmonella, with bacterial load at the peak of infection, and the primary T cell response being equivalent in B cell-deficient and wild-type mice (17). However, protective immunity is absolutely dependent on the presence of B cells, with B cell-deficient mice showing hugely impaired IL-2 and IFN-γ production by T cells after bacterial clearance (18), suggesting an absence of T cell memory (18, 19) and, in addition, showing increased mortality during challenge (18). Transfer of immune serum did not restore protection in B cell-deficient animals, suggesting one important role of B cells in this model is the presentation of Ag to T cells for the generation of memory (18). Indeed, to transfer protection to naive mice, both immune serum and T cells are required (20).

In our recent work, we have addressed the specific role of B cell TLR stimulation in Salmonella infection. Using mixed bone marrow chimeras in which the B cell compartment alone is deficient in MyD88 (MyD88<sup>−/−</sup>) reveals that primary T cell IFN-γ production during S. typhimurium infection is reduced, suggesting B cells play an important role as APCs in driving the early Th1 response (4) (T. Barr, S. Brown, and D. Gray, unpublished observations). Looking at Ab production, IgG2a/c is reduced in MyD88<sup>−/−</sup> mice during S. typhimurium infection (4), and this correlates with the impairment of T cell production of IFN-γ. Recent work, showing TLR4-mediated changes to splenic structure (21), has highlighted the potential of TLR signaling to affect cell migration. This study started with the observation that certain TLR ligands alter the expression of CD62L on B cells, and we characterize in this study how this affects their migration during infection.
CD62L (L-selectin) is an adhesion molecule belonging to the C-type lectin family that binds carbohydrate ligands, such as those induced on inflamed endothelium and those constitutively expressed at high endothelial venules (HEVs) (22). More specifically, CD62L ligands include sulfated carbohydrates of glycosylation-dependent cell adhesion molecule 1 and CD34 at lymph node HEVs (23) and mucosal addressin cell adhesion molecule-1 at Peyer’s patch HEVs (24). Binding of CD62L initiates tethering and rolling of cells and allows the subsequent transmigration from the bloodstream into tissues (25, 26). Blocking Abs against CD62L have been shown to inhibit lymphocyte binding to HEVs both in vitro and in vivo (27), whereas CD62L knockout (KO) mice display a 70–90% reduction in lymph node cellularity (28). Naïve-lymphocytes, including B cells, are CD62L+ and express varying levels of this molecule depending on the organ from which they are isolated. Upon stimulation by cognate Ag through the BCR or by phorbol esters such as PMA, CD62L is shed from the cell surface.

The enzyme responsible for shedding of surface CD62L is a zinc-containing membrane-associated metalloprotease, a disintegrin and metalloproteinase (ADAM) 17 (also known as TACE), which also cleaves TNF-α (29). This cleavage of CD62L by lymphocytes occurs rapidly, with 90% of lymph node cells shedding CD62L in response to PMA within 1 h (30).

CD62L expression is important in the development of immune responses, as CD62L-deficient mice have reduced leukocyte migration to inflamed sites and impaired delayed-type hypersensitivity responses (31), as well as impaired primary T cell proliferation and cytokine production (32). Furthermore, the metalloprotease-mediated shedding of CD62L is crucial, as mice expressing a mutant form that cannot be cleaved from the cell surface exhibited impaired responses to viral infection and delayed viral clearance (33, 34). All of the work to date on CD62L has focused on T cells, impaired responses to viral infection and delayed viral clearance (31), as well as impaired primary T cell proliferation

In vitro TLR stimulation cultures

Splenocytes or purified B cells were cultured at 4 × 10^6 cells/ml in complete IMDM in 24-well plates. Endotoxin-free TLR ligands (Invivo-Gen, Autogen Bioclear, U.K., Wiltshire, U.K.) were used at the following concentrations: the TLR2 ligands zymosan, peptidoglycan, and PAM2 CSK4 were used at 10, 10, and 0.2 μg/ml, respectively; the TLR3 ligand polyinosinic-polycytidylic acid (poly I:C) was used at 25 μg/ml; LPS (TLR4) from Escherichia coli was used at 1 μg/ml; flagellin (TLR5) from S. typhimurium was used at 0.1 μg/ml; the TLR7 ligand loxoribine was used at 100 μM; and the TLR9 ligand unmethylated CpG DNA (ODN 1826, 5’-TCC AGC ATG TTC GTG TT-3’) was used at 5 μg/ml. PMA and ionomycin (Sigma-Aldrich, Poole, U.K.) were used at 10 ng/ml and 1 μg/ml, respectively. Where appropriate, the metalloprotease inhibitor Ro 31-9790 (Roche Research Products, Welwyn Garden City, U.K.) was used at 50 μg/ml. For the recovery of CD62L expression, cells were harvested, washed, and plated out again at 4 × 10^6 cells/ml in fresh media.

In vivo CpG DNA immunization

C57BL/6 mice were immunized with 20 μg CpG DNA i.v. or mock immunized with PBS. Mice were bled preimmunization and at various times postimmunization. Lymphocytes were isolated by separation on lymphocyte (Cedarlane Laboratories, Hornby, Ontario, Canada) and washed extensively prestaining.

S. enterica serovar Typhimurium infection

The arsA-attenuated strain of S. typhimurium (SL3261) was used for all infections (39). Bacteria were grown as stationary-phase overnight (16 h) cultures in Luria-Bertani broth (Difco Laboratories, Surrey, U.K.). Animals were injected i.v. with 10^6 CFU diluted in PBS. Infectious dose was determined by plating bacteria onto Luria-Bertani plates and culturing overnight at 37°C.

Preparation of S. typhimurium Ags

Bacterial Ags from S. enterica serovar Typhimurium were prepared as previously described (40). Briefly, overnight stationary-phase cultures of the SL3261 strain were heat-inactivated at 85°C for 10 min; ~2.5 × 10^9 CFU bacteria that had undergone heat inactivation were used for in vitro cultures. For B cell culture with live bacteria, around 2.5 × 10^7 CFU bacteria was used. To prepare the crude sonicate, overnight-cultured bacteria (SL3261 strain) were sonicated and debris removed by centrifugation. To make the C5 Ag, overnight culture of the C5 virulent strain of S. typhimurium was sonicated, alkali treated (NaOH), and neutralized in HCl. Both the crude sonicate and C5 Ag were used at 20 μg/ml.

In vivo proliferation assay

Mice were injected with 2 mg BrdU i.p. on days 2, 4, and 8 of Salmonella infection. The following day (days 3, 5, and 9), spleens were removed and single-cell suspensions prepared. Proliferation, as determined by BrdU incorporation, was measured by staining for BrdU using an FITC-BrdU Flow Kit (BD Biosciences, San Jose, CA) as described in the manufacturer’s instructions. Briefly, cells were surface stained with anti-CD19 PE and anti-CD4 APC, washed, then resuspended in fix/perm buffer overnight. The following day, cells were washed and DNase treated for 1 h at 37°C, then stained with anti-BrdU FITC.

Cell transfers

For cultured cell transfer, purified B cells from Ly5.1+ donor mice were cultured with CpG DNA as described above for 4 h or left unstimulated. Postharvesting, cells were washed extensively in PBS and 5 × 10^6 cells injected i.v. into recipient C57BL/6 mice. The CD62L phenotype of CpG-stimulated cells was confirmed by flow cytometry.

For CD62L blood cell transfer, blood was collected into heparin from donor mice by cardiac puncture. Lymphocytes were extracted and washed as described above. A total of 5 × 10^7 unsorted lymphocytes was injected i.v. into recipient uninfected or day 6 Salmonella-infected mice. The CD62L phenotype of donor cells was confirmed by flow cytometry.

Flow cytometry

Prestaining, cells were washed in FACS buffer (PBS with 0.05% sodium azide and 3% FCS). The following Abs were used (all from BD Biosciences unless otherwise stated): anti-CD19-PE, anti-CD4-APC, anti-CD62L-FITC (Abcam, Cambridge, U.K.), IgG2a-FITC isotype control (Abcam), anti-Ly5.1-biotin, and streptavidin-PerCP. Cells were stained for 20 min on ice and washed three times in FACS buffer. Samples were analyzed on
an FACSCalibur flow cytometer (BD Biosciences) using CellQuest software and data analyzed using FlowJo software (Tree Star, San Carlos, CA).

Statistics
The Student paired t test was used to calculate significance values where appropriate.

Results
Loss of CD62L expression on B cells in response to some TLR ligands
B cells are known to lose expression of CD62L following the ligation of their BCR (41). We wished to know whether activation via innate receptors, such as TLRs, altered expression of this molecule. To examine the effect of TLR stimulation of B cells on their expression of CD62L, purified B cells were cultured with a variety of TLR ligands in vitro. We found that B cells lose expression of CD62L in response to specific TLR ligands, namely PAM3CSK4 (a TLR2 ligand), CpG DNA (TLR9 ligand), and partially shed in response to poly I:C (a TLR3 ligand). In relation to the latter (TLR3), increasing the dose of poly I:C caused a greater degree of CD62L loss (Supplemental Fig. 1), indicating the partial loss is simply a result of efficacy. Stimulation with zymosan, peptidoglycan (both TLR2 ligands), LPS, flagellin, or loxoribine (ligands for TLR4, -5, and -7, respectively) did not induce shedding (Fig. 1A).

The highest levels of CD62L expression are found on follicular B cells (Supplemental Fig. 1) and peripheral blood B cells (similar profile to follicular B cells; data not shown); however, even the low levels seen on marginal zone B cells are significantly modulated by CpG stimulation (Supplemental Fig. 1).

Using cells from MyD88−/−, TRIF−/−, TLR2−/−, and TLR9−/− mice, it was confirmed that loss of CD62L on B cells in response to PAM3CSK4 is MyD88 and TLR2 dependent, its loss in response to poly I:C is TRIF dependent, and, in response to CpG DNA, is MyD88 and TLR9 dependent. All KO B cells were able to shed when stimulated with PMA/ionomycin (Fig. 1B) or by cross-linking their BCR (data not shown).

Loss of CD62L expression by B cells stimulated with CpG DNA is rapid and due to shedding from the surface
An analysis of CD62L expression over time after CpG stimulation revealed that B cells lost surface expression of CD62L within 2 h of culture (Fig. 2A). This expression was not restored within the 12 h analyzed. To establish whether B cells lose CD62L expression in vivo in response to CpG DNA, mice were injected with 20 μg CpG DNA. As can be seen in Fig. 2B, the percentage of B cells in the blood that express high levels of CD62L was significantly reduced at 2, 4, and 8 h postinjection. Expression levels returned to normal by 24 h postinjection.

It seems likely that the mechanism by which B cells rapidly downregulate CD62L is by shedding from the surface, as characterized previously with respect to activation by PMA (33). To demonstrate that this was the case, we used the metalloprotease

**FIGURE 1.** B cells rapidly shed CD62L in response to some TLR ligands. Purified splenic B cells from C57BL/6 (A, B), MyD88−/−, TRIF−/−, TLR2−/−, and TLR9−/− mice (B) were cultured with a variety of TLR ligands for 4 h. B cell expression of CD62L was analyzed by flow cytometry. PMA/ionomycin is included as a positive control. In A, dotted line represents unstimulated, and bold line represents isotype control. In B, dashed line represents unstimulated, and bold line represents TLR stimulated. The data presented are from a single experiment using one spleen from each of the KO mice. Data are representative of four independent experiments.
inhibitor Ro 31-9790, which has previously been shown to inhibit CD62L shedding (42). Ro 31-9790 also inhibits shedding of TNF-α (43) and is, therefore, thought to target ADAM17 (TACE). When this inhibitor was included in the B cell culture with CpG DNA, the loss of CD62L from the cell surface at the 4-h time point was completely abolished (Fig. 2C). This confirms that the loss of surface CD62L by B cells in response to CpG DNA was due to shedding by the surface metalloprotease ADAM17 (TACE) and not any other mechanism.

CpG-stimulated B cells are excluded from lymph nodes and Peyer’s patches
It is well known that CD62L is essential for leukocyte entry into the lymph nodes across the HEV (27). We predicted, therefore, that CpG-stimulated B cells that have a CD62Llo phenotype would show altered migration patterns in vivo. To investigate this, we transferred CpG-stimulated or unstimulated B cells, after 4 h of culture, into congenic Ly5-distinct hosts and looked the following day at their localization. The CpG-stimulated CD62Llo donor cells were ex-
cluded from the lymph nodes and Peyer’s patches (Fig. 3) but trafficked normally to the spleen. These data indicate that TLR stimulation of B cells can impact on their migration patterns in vivo via changes in surface expression levels of CD62L.

**B cells recover surface expression of CD62L ∼ 2 to 3 d poststimulation and subsequently gain entry into lymph nodes and Peyer’s patches.**

Having established the kinetics of CD62L shedding from the surface of B cells in response to TLR stimulation, we next analyzed the recovery of expression both in vitro and in vivo. Following culture with CpG DNA, B cells were washed extensively and either plated out again with fresh media in vitro or adoptively transferred into Ly5-distinct recipient mice. Fig. 4A shows that B cells recover CD62L expression after 3 d in culture, and in vivo, the re-expression happens after 2 d (Fig. 4B). The adoptive transfer showed that donor CpG-activated B cells were present in the lymph nodes and Peyer’s patches by day 5 in numbers equivalent to unstimulated B cells. The lag of 3 d between CD62L re-expression in the spleen on day 2 and B cell migration to lymph nodes and Peyer’s patches on day 5 is likely due to the normal homing patterns of B cells, in that they pause in secondary lymphoid tissues before re-entering the circulation.

**B cells shed CD62L when stimulated with Ags from Salmonella.**
To confirm that B cells shed CD62L when encountering TLR ligands in the form of pathogenic bacteria, we stimulated B cells with Ags from the Gram-negative bacteria *S. enterica* serovar Typhimurium (hereafter referred to as *S. typhimurium*). The data presented in Fig. 5A reveal that B cells shed CD62L when stimulated with heat-killed bacteria, a crude sonicate of bacteria, or a semi-purified C5 Ag. However, no shedding occurred in response to live bacteria in vitro.

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**FIGURE 4.** Pattern of CD62L recovery by B cells both in vitro and in vivo. B cells were stimulated with CpG DNA for 4 h in vitro. **A.** Cells were harvested, washed, and plated out again. Samples were taken up to 4 d later and analyzed for B cell expression of CD62L. **Left panels,** unstimulated; **right panels,** CpG-stimulated. B. After washing, 5 million cells were transferred into Ly5-distinct mice. Over the following days, organs were removed, donor cells identified, and CD62L expression levels analyzed. Error bars represent SEM with four mice per group. Data are representative of three experiments.
To determine if a specific TLR and adaptor molecule is responsible for shedding induced by these bacterial Ags, KO cells were used. The data in Fig. 5 show that TLR2 and the adaptor molecule MyD88, rather than TLR9 or TRIF, are used to induce shedding in response to these bacterial Ag preparations.

**Altered localization of B cells during S. typhimurium infection**

The data shown so far would predict that TLR-induced changes in CD62L expression will result in altered migration of B cells during systemic infection with *S. typhimurium*. The data presented in Fig. 6A and 6B show that *S. typhimurium* infection caused profound alterations in the localization of B cells. In the lymph nodes and Peyer’s patches, there was a rapid decrease in both the percentages (Fig. 6A) and absolute numbers (Fig. 6B) of B cells during the first week of infection. This was most apparent in the Peyer’s patches, in which the proportion of B cells dropped from \( \sim 80\% \) in an uninfected mouse to 20\% by day 8 of infection. In the spleen, the percentage of B cells remains unchanged at day 4 and is reduced at day 8 (Fig. 6A), largely due to an influx of other cells (e.g., macrophages). However, looking at absolute numbers (Fig. 6B), there is actually a 2- to 3-fold increase in splenic B cell numbers at day 4 when compared with an uninfected mouse, and numbers remain elevated at day 8. The increase in B cell numbers in the spleen at this early stage of infection does not seem to be due to B cell proliferation, as BrdU incorporation by B cells was little above background and minimal in comparison with T cell division (Fig. 6C, 6D).

The changes in B cell populations seen in lymphoid organs (increase in spleen, reduction in lymph nodes and Peyer’s patches) during *Salmonella* infection are likely due to TLR activation, as they are not so apparent in MyD88\(^{-/-}\) mice (Supplemental Fig. 2).

These changes in B cell distribution within lymphoid organs are long lasting (Supplemental Fig. 3B). This is likely due to the chronic nature of this infection, in which bacteria were not cleared until 6–8 wk postinfection (Supplemental Fig. 3A). Together, the data in Fig. 6 support the notion that B cells shed CD62L in response to bacteria, are excluded from lymph nodes, and consequently enter the spleen in greater numbers, as entry to the spleen is independent of CD62L (44).

**CD62L\(^{hi}\) B cells shed and migrate to the spleen, not lymph nodes, when transferred into *Salmonella*-infected mice**

The data shown so far suggest that B cells shed CD62L during *S. typhimurium* infection and that this is the cause of altered migration patterns. To address this more directly, we isolated CD62L\(^{hi}\) B cells from the blood of Ly5.2\(^{+}\) donor mice and transferred them into either *S. typhimurium*-infected or uninfected Ly5.1\(^{-}\) recipient mice. Ly5.2\(^{+}\) donor B cells were identified and CD62L expression analyzed after 18 h. The results displayed in Fig. 7 demonstrate that in uninfected mice, donor Ly5.2\(^{+}\) cells migrated into both the spleen and lymph nodes, whereas in infected mice, the Ly5.2\(^{+}\) B cells were found in much greater numbers in the spleen and significantly reduced numbers in the lymph nodes. Donor cells identified in the spleens of uninfected mice have maintained their CD62L\(^{hi}\) phenotype, whereas in the spleens of infected mice, donor B cells have shed and are predominantly CD62L\(^{lo}\) (Fig. 7C). These data confirm that B cells shed CD62L early during *S. typhimurium* infection and that...
this is related to their enhanced entry into the spleen and relative exclusion from the lymph nodes and Peyer’s patches.

Discussion
The data presented in this study indicate that stimulation of B cells through TLR2, -3, and -9 induces shedding of CD62L, which impacts on their migration patterns and results in their exclusion from lymph nodes and Peyer’s patches. During S. typhimurium infection, this process causes B cells to accumulate in the spleen during the first week. We found that only certain TLR ligands (PAM3CSK4, poly I:C, and CpG-DNA) induced shedding of CD62L by B cells in vitro (Fig. 1A). Interestingly, PAM3CSK4, which binds TLR2/1 heterodimers, induced shedding, whereas other TLR2 ligands, zymosan and peptidoglycan, which bind TLR2/6 heterodimers, had no such effect. Differences with the zymosan signal could be explained by its dependence on dectin 1 (45), which is not expressed by B cells (46). The reason is more likely to be due to differential expression of TLR1 and TLR6 by B cells. There is evidence to suggest that although B cells express TLR6 at the mRNA level (1, 2), they display a greater proliferative response to TLR2/1 stimulation than to TLR2/6 (2), which would support the notion of increased expression of TLR1 compared with TLR6. Therefore, although these two forms of TLR2 signal via the same pathway and so produce

FIGURE 6. B cell localization is altered during S. typhimurium infection. C57BL/6 mice were infected with the attenuated SL3261 strain of S. typhimurium. Spleens, mesenteric lymph nodes, and Peyer’s patches were taken at various times during infection and percentages (A) and absolute numbers (B) of B cells identified. Some mice were injected (at either day 2, 4, or 8) with 2 mg BrdU i.p. and splenocytes analyzed the following day for percentages (C) and absolute numbers (D) of proliferating cells. Error bars indicate SEM with four mice per group.
the same effects (47), differential expression would account for the differences seen between these stimuli.

Shedding of CD62L by B cells in response to TLR stimulation has not previously been reported. However, other groups have indicated that human neutrophils rapidly lose expression of CD62L following stimulation with TLRs in vitro (48, 49), and this method has been used to detect defects in the TLR signaling pathway (48). However, these two articles do not agree on which TLRs induce shedding. On the one hand, Von Bernuth et al. (48) suggested that shedding of CD62L by human neutrophils was stimulated by ligands for all of the TLRs expressed by granulocytes (TLR2/1, -2/6, -4, -5, -7, and -8). On the other hand, Hayashi et al. (49) only saw neutrophil shedding of CD62L in response to TLR2/1, -2/6, -4, and -7/8 but not TLR3, -5, or -9. The differences seen between these results in relation to TLR5-induced shedding may be due to different mechanisms of extracting neutrophils or the varying concentrations of flagellin used. However, these two articles do not agree on which TLRs induce shedding.

On the one hand, Von Bernuth et al. (48) suggested that shedding of CD62L by human neutrophils was stimulated by ligands for all of the TLRs expressed by granulocytes (TLR2/1, -2/6, -4, -5, -7, and -8). On the other hand, Hayashi et al. (49) only saw neutrophil shedding of CD62L in response to TLR2/1, -2/6, -4, and -7/8 but not TLR3, -5, or -9. The differences seen between these results in relation to TLR5-induced shedding may be due to different mechanisms of extracting neutrophils or the varying concentrations of flagellin used. However, these two articles do not agree on which TLRs induce shedding.

The TLRs that induce shedding of CD62L by B cells in response to bacteria (TLR2, -3, and -9) are not the most abundant or obvious TLR ligands to have an effect during Salmonella infection. Although the immune roles of LPS (TLR4) and flagellin (TLR5) in the Salmonella infection model have been investigated (50, 51), the TLRs highlighted in this study have received less attention. Interestingly, it has been noted that infection with S. typhimurium results in an increase in expression of TLR1, -2, and -9 in the infected liver (52), but the spleen was not investigated. Furthermore, incubation of hepatocytes with CpG DNA significantly inhibited the intracellular growth of S. typhimurium in vitro, suggesting that stimulation via TLR9 can enhance bacterial killing (53). Rumio et al. (54) also demonstrated that pretreatment with CpG-DNA for 72 h in vivo increases survival when mice are then infected with virulent S. typhimurium, again suggesting a role for this TLR in inducing bacterial killing. The authors propose that this increased survival is due to the responses of Paneth cells, although they did not investigate this directly. This effect may be partly due to the...
TLR9-induced changes in B cell localization and activation. How B cells would be activated by bacterial DNA during infection remains to be clarified, as it seems that unless it is released as soluble material following bacterial cell death, the bacterial particles would require receptor (BCR)-mediated uptake (55, 56), and so, only Salmonella-specific B cells might be activated via TLR9.

The TLR-induced shedding of CD62L by B cells had a profound impact on their migration in vivo. These TLR-stimulated B cells are completely excluded from the lymph nodes and Peyer’s patches and traffic only to the spleen (Fig. 3). Our data in this study suggest that, following a single stimulation with TLR ligands, B cell CD62L returns to normal levels in 2 to 3 d, and these cells are then able to traffic normally to the lymph nodes and Peyer’s patches (Fig. 4). Others have shown that, in T cells, activation by anti-CD3 induces a similar short-term reduction in surface CD62L, with normal levels achieved 2 to 3 d later (57, 58). This is followed 7 d later by full activation of T cells and downregulation of surface CD62L as a result of transcriptional regulation (58). In the Salmonella infection model, we see that the reduction of B cell numbers in the lymph nodes and Peyer’s patches is only apparent for the first 8 d of infection, and thereafter, B cell numbers in these organs begin to return to normal. In the spleen, B cell numbers are elevated on days 4 and 8, but not from day 12 onwards. This would suggest that the focusing of B cells to the spleen by TLR-induced changes in CD62L expression, as seen in Salmonella infection, is a short-term phenomenon.

The data presented indicate that, during Salmonella infection, TLR stimulation of B cells induces a short-term reduction in levels of surface CD62L, which in turn results in increased B cell trafficking to the spleen (Fig. 6). This may be a mechanism to nonspecifically attract a polyclonal population of B cells to the spleen. We propose that this will have two effects. First, it will enhance the BCR-dependent activation/selection of Ag-specific B cells in the spleen, both by exposing greater numbers of B cells to bacterial Ags but also by TLR-mediated augmentation of expression of MHC class II and costimulatory molecules (59–61). Second, the subsequent polyclonal activation by these and other TLR ligands will drive B cell cytokine production (1). This cytokine release by B cells has a significant effect on the programming of the early, primary CD4 T cell response (T. Barr, S. Brown, and D. Gray, unpublished observations). In addition, this nonspecific accumulation of B cells in the spleen may enhance the secretion of natural Ab directly to the site where the bacteria have amassed. Therefore, TLR-induced IgM production in the spleen could bind to the bacteria and contribute to the initiation of the primary response as has been reported (62, 63) and ultimately enhance bacterial killing.

Whether the TLR/CD62L-mediated changes to B cell migration enhance the initiation of the adaptive immune response to Salmonella or whether TLR-activated B cells may also play roles in the regulation of the inflammatory response (64–66) or changes to the lymphoid tissue structure during infection (21, 67), the global change in migration behavior upon TLR ligation seems likely to have significant consequences for the immunopathology of this infection.

Disclosures
The authors have no financial conflicts of interest.

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